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Dear Sir:

Since 1927 we have been poisoning animals to death in lethal toxicity tests. And we have been doing so knowing that if a person were to do the same thing to an animal outside of a laboratory, he or she could be arrested and charged with cruelty to animals.

Starting in the 1980s, Bjorn Eckwall begged the scientific community to consider his methodology using cell death as a more accurate (and obviously more humane) alternative. In the late 1990s, the U.S. EPA and The Johns Hopkins Center for Alternatives to Animal Testing (CAAT) exhibited criminal disinterest in regards to this new technology. I can personally attest to this fact because Mary Beth Sweetland, PETA's Director of Research and Investigations, and I begged both the EPA and CAAT to take a look at Dr. Eckwall's data and were greeted with total indifference and, indeed, hostility.

Largely as a result of our negotiations with the White House, which resulted in minimal protections for animals used in the newly proposed EPA High Production Volume (HPV) chemical-testing program, ICCVAM was tasked with putting together an international workshop to garner expert opinion on the feasibility of using cytotoxicity studies to (a) reduce the number of animals killed in lethal dose testing and (b) completely replace lethal dose testing on animals. This workshop was held in October 2000.

Despite near unanimous agreement amongst the experts at that workshop that the cell-based methods could be used immediately to reduce the numbers of animals killed and that, within a few years – given the proper funding and effort – the method could be validated as a replacement measure,[1] the organizers of that workshop cynically and blatantly ignored the second half of those recommendations, as represented here from Figure 2.6 of the report:[2]

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[1] Report of the International Workshop on In Vitro Methods for Assessing Acute Systemic Toxicity, NIH Pub. No. 01-4499, p. 31: "It was considered that, if the commitment to conducting a formal validation study was strong enough…a replacement test battery might be achieved in as short a time as 2-3 years."
[2] *Op. cit.*

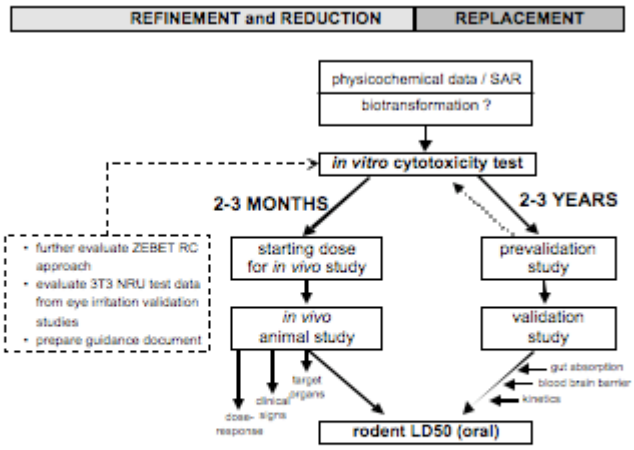

Figure 2.6. Strategy for the reduction, refinement and replacement of animals in acute LD50 testing

Around the same time, ICCVAM also issued a "Guidance Document on Using *In Vitro* Data to Estimate *In Vivo* Starting Doses for Acute Toxicity."[3] To further the goal of validating the cytotoxicity tests as eventual replacement methods, EPA was prevailed upon to issue guidance to the participants of the HPV program. This guidance asked that sponsors use the *in vitro* method to set the starting dose if acute toxicity tests were to be conducted under the program:[4] "The October 2000 Workshop concluded that *in vitro* cytotoxicity data could be useful in estimating starting doses for *in vivo* acute toxicity testing, and in this way could also reduce the number of animals used in subsequent *in vivo* tests."

Further, a standard test reporting template was provided,[5] ostensibly to collect data from the tests' use to set starting doses and to help validate them as replacement methods. In February 2001, the EPA and NIEHS held another workshop to help familiarize the scientific community with these methods.

A quick review of the tests proposed and conducted under the HPV program shows that, of the approximately 20 acute systemic toxicity tests conducted and currently pending, only one cytotoxicity test was apparently conducted to set the starting dose. This 0.05 rate of cytotoxicity use is a disappointing figure, especially given all the government hype that the HPV program would be helpful in collecting data to facilitate the validation of non-animal test methods.

[3] NIH Pub. No. 01-4500
[4] http://www.epa.gov/oppt/chemrtk/toxprtow.htm
[5] http://www.epa.gov/chemrtk/nvtrotmp.pdf

The current ICCVAM workshop is taking place more than 5 ½ years later for the stated purpose of studying "the use of *in vitro* testing methods for estimating starting doses for acute oral systemic toxicity tests."[6] There is no mention of the potential use the cytotoxicity tests as an eventual replacement method. To the best of our knowledge, no additional prevalidation studies of any pharmacodynamic models were initiated while the larger cytotoxicity study was in progress nor were any of the intermediate-term activities, recommended in the 2000 workshop report, implemented: "Continued development and optimization of such systems [as gut absorption, BBB passage, key kinetic parameters, and metabolism] for this application should be encouraged and should receive regulatory support."[7] This complete failure, on the part of the government entity charged with coordinating the acceptance of non-animal testing methods, to make any progress on such a critical issue is a disgrace.

In addition to taking a tremendous amount of time to complete, the ICCVAM study had a basic problem with defining a prediction model. Early on, the German government alternatives group, ZEBET, informed the study organizers that the chemicals selected for the study were highly biased towards under-predicted materials. ZEBET pointed out that this fact would make the study difficult to interpret and would likely result in an incorrect description of the prediction equation: "With these test chemicals [selected], the study outcome can only be a falsification of the prediction model."[8] To our knowledge, these concerns were never addressed and ICCVAM forged ahead, knowing that it was using outliers which would only serve to invalidate the method.

In 2001, PETA submitted a letter to Dr. Stokes, raising a number of inconsistencies with regard to ICCVAM's approach to the cytotoxicity validation studies (attached). It has been clear from the beginning that ICCVAM has no interest in advancing the use of the cytotoxicity tests as a replacement method for one of the – if not the most – cruel tests in use today. While the results of ICCVAM's insistence on ignoring a potential scientific and humane breakthrough are clear, the reason for this enmity remains unknown.

Then-acting deputy director of NTP, Chris Portier, as well as others, have expressed their beliefs that, because the acute systemic toxicity endpoint is not a worthwhile endpoint, it is not worth investing much effort into developing a better way to measure it. But this stance does not change the fact that the acute systemic toxicity endpoint is still a commonly required test by the U.S. government. So while we may not consider it to be a scientifically useful endpoint, regardless of whether it is performed *in vivo* or *in vitro*, the fact remains that animals continue to be poisoned to death to test this endpoint.

The question that remains is why the recommendations and plan agreed upon more than 5 ½ years ago at ICCVAM's own workshop were not followed in the manner outlined. This debacle is not all that dissimilar from ICCVAM's insistence on confirmatory testing for Epiderm results for determination of corrosivity. And while the Europeans have

[6] Federal Register, Vol. 71, No. 54, 3-21-06
[7] Report of the International Workshop on In Vitro Methods for Assessing Acute Systemic Toxicity, NIH Pub. No. 01-4499, p. 33.
[8] 3-1-04 letter from ZEBET to Dr. Stokes

developed and validated a number of replacement methods, including for photoxicity and pyrogenicity testing on animals, we have yet to see any movement in these areas in the U.S.

We are not asking ICCVAM to be a blind advocate for non-animal methods but to demonstrate objectivity and to further the goal of good science. It is not good science – nor is it objective – for ICCVAM to require the use of a non-validated animal test to confirm the negative results of a validated non-animal method (as it has done on the Epiderm/corrosivity issue mentioned above). Sadly, ICCVAM's refusal to focus on replacing the use of animals in lethal dose testing now joins the dismal list that demonstrates ICCVAM's continued disregard for its Congressional mandate

Sincerely,

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